



Importance of the B2 domain of the *Arabidopsis* ABI3 protein for Em and 2S albumin gene regulation

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Abstract

Genetic and molecular studies have shown that the *Arabidopsis* ABSCISIC ACID-INSENSITIVE3 (ABI3) protein plays a prominent role in the control of seed maturation. The ABI3 protein and its orthologues from various other plant species share four domains of high sequence identity, including three basic domains designated as B1, B2 and B3. The leaky *abi3-1* mutation is a single amino acid substitution within the B3 domain. A new *abi3* allele, *abi3-7*, was generated by mutagenizing *abi3-1* seeds. The *abi3-7* line contains, in addition to the *abi3-1* mutation, a point mutation that converts residue Ala-458 into Thr within the B2 domain of the ABI3 protein. This Ala residue is absolutely conserved in all known ABI3 orthologues. *Abi3-7* seeds display reductions in dormancy and in sensitivity to abscisic acid which are intermediate between those of the leaky *abi3-1* and of the severe *abi3-4* and *abi3-5* mutants. Accumulation and distribution of *At2S1* and *At2S2* albumin mRNA as well as of *AtEm1* and *AtEm6* late embryogenesis-abundant proteins and mRNA have been analyzed. Both *At2S1* and *At2S2* mRNA are reduced in *abi3-7*, but distribution of *At2S2* is spatially restricted. Accumulation of *AtEm6* protein is more sensitive to *abi3-7* mutation than *AtEm1*. However both mRNAs are considerably reduced in this mutant. Their distribution is also differentially affected. These results provide genetic evidence for the importance of the conserved B2 domain for ABI3 function *in vivo*.

Introduction

In *Arabidopsis*, genetic studies have demonstrated that the *abscisic acid-insensitive 3* (*ABI3*) gene plays a prominent role in multiple facets of embryo maturation. Seeds of severe *abi3* mutants display a marked reduction in sensitivity to ABA, remain non-dormant, fail to degrade chlorophyll, do not acquire desiccation tolerance, and accumulate reduced levels of many storage proteins and late-embryogenesis-abundant (LEA) proteins (Finkelstein and Somerville, 1990; Nambara *et al.*, 1992; Finkelstein, 1993; Ooms *et al.*, 1993;

Parcy *et al.*, 1994). Mutations in the *viviparous 1* (*VPI*) gene from maize lead to similar phenotypic defects (McCarty *et al.*, 1989, 1991; Kriz *et al.*, 1990; Pla *et al.*, 1991) and molecular analysis indicates that *ABI3* is orthologous to *VPI* (McCarty *et al.*, 1991; Giraudat *et al.*, 1992). Closely related genes have subsequently been isolated from many additional monocotyledonous and dicotyledonous species such as *Oryza sativa* (Hattori *et al.*, 1994), *Phaseolus vulgaris* (Bobb *et al.*, 1995), *Craterostigma plantagineum* (Chandler and Bartels, 1997), *Hordeum vulgare* (Hol-

lung *et al.*, 1997), *Avena fatua* (Jones *et al.*, 1997) and from *Populus trichocarpa* (Rhode *et al.*, 1998).

All these genes encode for proteins sharing four domains of high amino acid sequence identity: the A1 domain located in the large acidic N-terminal region, and three basic domains designated as B1, B2 and B3 in order from the N-terminus (Jones *et al.*, 1997; Rhode *et al.*, 1998). The acidic N-terminal region, containing the A1 domain, has a transcriptional activation function (McCarty *et al.*, 1991; Hattori *et al.*, 1994; Bobb *et al.*, 1997). *Arabidopsis* and maize mutant alleles, encoding truncated ABI3 or VP1 proteins that lack both the B2 and B3 domains, such as *abi3-4* (Giraudat *et al.*, 1992; Parcy *et al.*, 1994) and *vp1-mum1* (McCarty *et al.*, 1989; Carson *et al.*, 1997) have severe and pleiotropic phenotypic alterations, suggesting that the B2 and/or B3 domains are required for the regulation of seed maturation. Evidence that the B3 domain is essential for at least a subset of the ABI3/VP1 roles in seed maturation came from the characterization of the *abi3-1* mutant carrying a single amino acid substitution in the ABI3 B3 domain (Finkelstein and Somerville, 1990; Finkelstein, 1993; Ooms *et al.*, 1993; Parcy *et al.*, 1997), and of the *vp1-McW* mutant allele predicted to encode a truncated VP1 protein that lacks most of the B3 domain (McCarty *et al.*, 1989; Carson *et al.*, 1997). In contrast, the lack of appropriate mutant alleles has thus far impeded the analysis of the functional importance of the B2 domain for ABI3/VP1 action *in vivo*.

We report here the isolation of a novel *abi3* mutant allele, which carries a single amino acid substitution in the conserved B2 domain. This mutation is shown to differentially affect the expression of various members of the 2S *Albumin* and *Em* gene families during seed maturation.

Materials and methods

Plant material and germination assays

All the *Arabidopsis* lines used derive from the Landsberg *erecta* ecotype except *abi3-3* which is in the Columbia background. *abi3-1* (isolate name CIV), *abi3-4* (isolate name SMI) and *abi3-5* (isolate name 10286) have been isolated and described by Koornneef *et al.* (1984) and Ooms *et al.* (1993). *abi3-3* has been isolated by Nambara *et al.* (1992). The mutant line *abi3-7* was generated from *abi3-1* seeds treated with 15 mM ethyl methane sulfonate for 24 h. Plants were

routinely grown on soil, in a greenhouse (18–22 °C) with additional light (Philips TLD 58W/84, 16 h light period). For germination assays seeds were sown on filter papers imbibed with water or an ABA solution and incubated for 7 days at 25 °C with a 16 h light period.

Protein extraction and western blot analysis

For ABI3 immunodetection, seeds were excised from dry siliques, counted, and immediately frozen in liquid nitrogen. Total proteins were extracted using a previously described phenol extraction method (Meyer *et al.*, 1988). Dried pellets were solubilized in 1× SDS gel-loading buffer (Sambrook *et al.*, 1989), the volume of buffer was adjusted to the number of seeds. Proteins were fractionated by electrophoresis on 8% SDS-polyacrylamide gels and then electroblotted onto nitrocellulose membranes (Sambrook *et al.*, 1989). Immunological detection of the ABI3 protein was performed by an enhanced chemiluminescence method (Amersham) using as primary antibodies a polyclonal antiserum raised against the N-terminal domain of the ABI3 protein (Parcy *et al.*, 1994). For immunodetection of the Em proteins a technique already described (Bies *et al.*, 1998) was used. Protein extracts were prepared from seeds harvested at the same developmental stage as for ABI3 immunodetection. The antiserum used as primary antibodies was the polyclonal antiserum 6 which cross-reacts with both AtEm1 and AtEm6 *Arabidopsis* proteins (Bies *et al.*, 1998).

RNA blot analysis and in situ hybridizations

Total RNAs were extracted either from siliques harvested at 12 days after pollination (DAP) or from dry seeds and subjected to northern analysis as previously described (Bies *et al.*, 1998). *In situ* hybridizations were carried out as previously described by da Silva Conceicao and Krebbers (1994). *At2S1* and *At2S2* mRNAs were detected using the gene-specific probes FG1 and FG2, respectively (Guerche *et al.*, 1990). *AtEm1* and *AtEm6* mRNA were detected using probes obtained by *in vitro* transcription from the T7 or T3 promoter sequences located on either side of cDNAs inserts cloned as described by Gaubier *et al.* (1993). The complete *AtEm1* or *AtEm6* cDNAs were used as probes since they do not cross-hybridize. The 18S rRNA probe used to reflect RNA quantity loaded has been described by Chory *et al.* (1989).

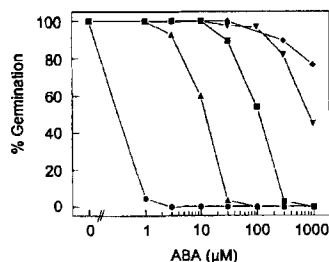


Figure 1. Germination capacity in the presence of ABA. Freshly harvested seeds from mature siliques of wild-type *Landsberg erecta* (●), *abi3-1* (▲), *abi3-4* (▼), *abi3-5* (◆) and *abi3-7* (■) mutants were chilled for 4 days at 4 °C in darkness to break dormancy, and then tested for their capacity to germinate in the presence of different concentrations of ABA. Data are averages \pm SD of triplicate assays, each with 40 to 80 seeds. The error bars were smaller than the symbol sizes.

DNA sequencing

Overlapping genomic fragments encompassing the entire *ABI3* gene were obtained from wild type (*Landsberg erecta*), *abi3-5* and *abi3-7* DNA by polymerase chain reaction (PCR) and the amplified products were sequenced directly on both strands using appropriate primers. Double-stranded DNA was sequenced on an Applied Biosystems (Foster City, CA) automated DNA sequencer (model 373A). Sequence analyses and alignments were done with programs of the Genetics Computer Group (Madison, WI) software package (Devereux *et al.*, 1984).

Results

Isolation of a new *abi3* allele: *abi3-7*

Seeds homozygous for the leaky *abi3-1* mutation were mutagenized with ethylmethane sulfonate and the derived M2 population was screened for enhancers of *abi3-1*. A mutant line was selected as being able to germinate in the presence of 100 μ M ABA, a concentration that inhibits germination of both wild-type and *abi3-1* seeds. As shown in Figure 1, this mutant line (subsequently named *abi3-7* when found to be a new *abi3* allele; see below) displays a degree of ABA insensitivity which is intermediate between those of the leaky *abi3-1* and of the severe *abi3-4* and *abi3-5* mutants.

In addition to its intermediate ABA insensitivity, the *abi3-7* mutant is also intermediate in its seed germination behaviour as shown in Figure 2. The capacity

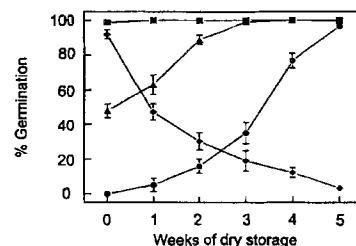


Figure 2. Germination behaviour in the absence of cold pretreatment. Freshly harvested seeds from mature siliques of wild-type *Landsberg erecta* (●), *abi3-1* (▲), *abi3-5* (◆) and *abi3-7* (■) mutants were tested for their ability to germinate without chilling pretreatment. Data are average \pm SD of triplicate assays, each with 40 to 80 seeds.

of freshly harvested seeds to germinate without cold treatment indicates the absence or reduction of seed dormancy. For wild-type seeds the dormancy rapidly declines after dry storage (after ripening). *abi3-5* seeds are not dormant but lose their viability within 5 weeks of storage at room temperature. *abi3-1* seeds have a reduced dormancy compared to wild-type and *abi3-7* seeds are not dormant and also not altered in their viability.

ABA sensitivity of F_3 progeny derived from the cross *abi3-7* \times WT (Ler) showed similar results in the presence of 10 and 100 μ M of ABA. A total of 26 progeny did not germinate, 14 germinated and 53 segregated (1/4 of the seeds germinated) indicating that one recessive mutation which could not be separated from the *abi3-1* allele (germination on 10 μ M ABA) was responsible for the ability of the seeds to germinate in the presence of 100 μ M of ABA. Whereas seeds of severe *abi3* mutants remain green at maturity (Nambara *et al.*, 1992, 1994, 1995; Ooms *et al.*, 1993), *abi3-7* seeds are only slightly greener than wild-type and *abi3-1* seeds. However, F_1 seeds derived from a cross between *abi3-7* and the severe *abi3-5* mutant were greener than F_1 seeds derived from a cross between the leaky *abi3-1* and the *abi3-5* mutant suggesting again that the enhancement of the *abi3-1* phenotype in *abi3-7* was due to an additional mutation within the *ABI3* gene.

To confirm this hypothesis the *ABI3* gene of the *abi3-7* mutant was sequenced and this analysis showed that *abi3-7* contains, in addition to the initial *abi3-1* mutation, a G-to-A transition at position 1777 in the cDNA insert of pcabi3-4F (GenBank accession number X68141) which converts Ala-458 to Thr in the *ABI3* protein. As shown in Figure 3, the latter amino acid substitution occurs within the conserved domain

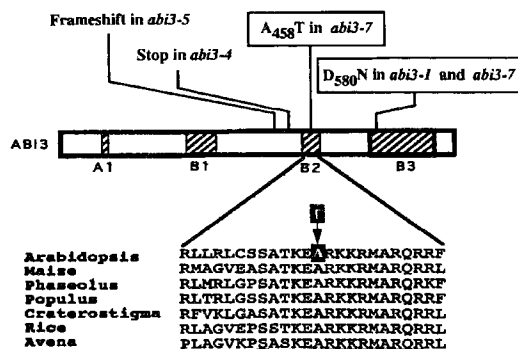


Figure 3. The *abi3* mutations. The upper part of the figure is a schematic diagram of the architecture of the ABI3 protein showing the four domains of high amino acid identity between ABI3 and its homologues from other plant species. The position of several *abi3* mutations is also shown on this diagram. The mutation *abi3-4* has been described by Giraudat *et al.* (1992) and *abi3-1* by Percy *et al.* (1997). The *abi3-5* mutation, which was induced by diepoxybutane (Ooms *et al.*, 1993), is a single-base deletion at position 1588 in the cDNA insert of pcabi3-4F (GenBank accession number X68141). In this mutant, translation of the ABI3 protein is predicted to continue in a wrong reading frame after residue Pro-394, between domain B1 and B2, resulting in the addition of 34 erroneous amino acids before a stop codon is encountered. The lower part of the figure is an alignment of the amino acid sequences of the B2 domain of the ABI3 homologues from various plant species. The residue Ala-458 converted into Thr in *abi3-7* is marked by a black square, and residues that are absolutely invariant in all the species are underlined.



Figure 4. Immunoblot analysis of ABI3 protein content. A 8% SDS-polyacrylamide gel was loaded with total protein extracts equivalent to 60 mature seeds from wild-type *Landsberg erecta* (lane 1), *abi3-1* (lane 2), and *abi3-7* (lane 3). Immunodetection was performed with an immune serum raised against the N-terminal domain of the ABI3 protein.

B2, and affects a residue that is absolutely invariant in ABI3 homologues from all plant species analysed thus far.

To determine if the additional point mutation in *abi3-7* affects the accumulation of ABI3 proteins and therefore could explain the intermediate phenotype a western blot analysis was carried out. The results reveal that ABI3 content in *abi3-7*, wild-type and *abi3-1* dry seeds are similar (Figure 4). This observation indicates that the *abi3-7* phenotype, compared to that of *abi3-1*, does not result from an instability of ABI3. All the subsequent analyses of *abi3-7* were performed using homogeneous progeny able to germinate in the presence of 100 μ M of ABA.

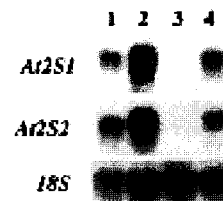


Figure 5. Northern blot analysis of *At2S1* and *At2S2* mRNA contents. *At2S1* and *At2S2* gene-specific probes were independently hybridized with total RNA (10 μ g) isolated from developing siliques harvested at 12 DAP from wild-type *Landsberg erecta* (lane 1), *abi3-1* (lane 2), *abi3-4* (lane 3) and *abi3-7* (lane 4) plants. The 18S rRNA control hybridization indicated that less RNA has been loaded in lane 1 and that the three other lanes are directly comparable. At 12 DAP expression of albumin genes is maximal (Guerche *et al.*, 1990).

Expression of *At2S1* and *At2S2* albumin genes

Mutant *abi3* seeds accumulate reduced amounts of storage proteins, and in particular of 2S albumins (Finkelstein and Somerville, 1990; Nambara *et al.*, 1992; Percy *et al.*, 1994). In *Arabidopsis*, the 2S albumins are encoded by a small gene family (Krebbbers *et al.*, 1988; van der Klei *et al.*, 1993). We analysed the impact of the *abi3-7* mutation on the regulation of two *Albumin* genes, *At2S1* and *At2S2*, that show differential expression patterns in the wild type (Guerche *et al.*, 1990).

Northern blot analysis showed that the *abi3-1* mutation has only a minor effect, if any, on the abundance of *At2S2* mRNA in developing seeds (Figure 5). In contrast, the *At2S2* mRNA content was significantly lower in *abi3-7* seeds than in *abi3-1*, although this reduction was not as pronounced as in the severe *abi3-4* mutant (Figure 5). To investigate whether the reduced *At2S2* mRNA content in *abi3-7* results from an overall reduction of *At2S2* promoter activity or from a restriction in *At2S2* tissue specificity several *in situ* hybridizations were performed. In agreement with a previous report (Guerche *et al.*, 1990), the *At2S2* mRNA was strongly detected in all the tissues of wild-type embryos (Figure 6a). A similar expression pattern was observed in *abi3-1* embryos (Figure 6b). In contrast, in *abi3-7* embryos, the *At2S2* mRNA was mainly detected in epidermal cells, the radicle of the embryo axis, and the shoot apical meristem (Figure 6c and 6d). This observation indicates that in *abi3-7*, the Ala-458-to-Thr substitution in the B2 domain of the ABI3 protein entails a restriction of the spatial pattern of *At2S2* expression. This restriction might be responsi-

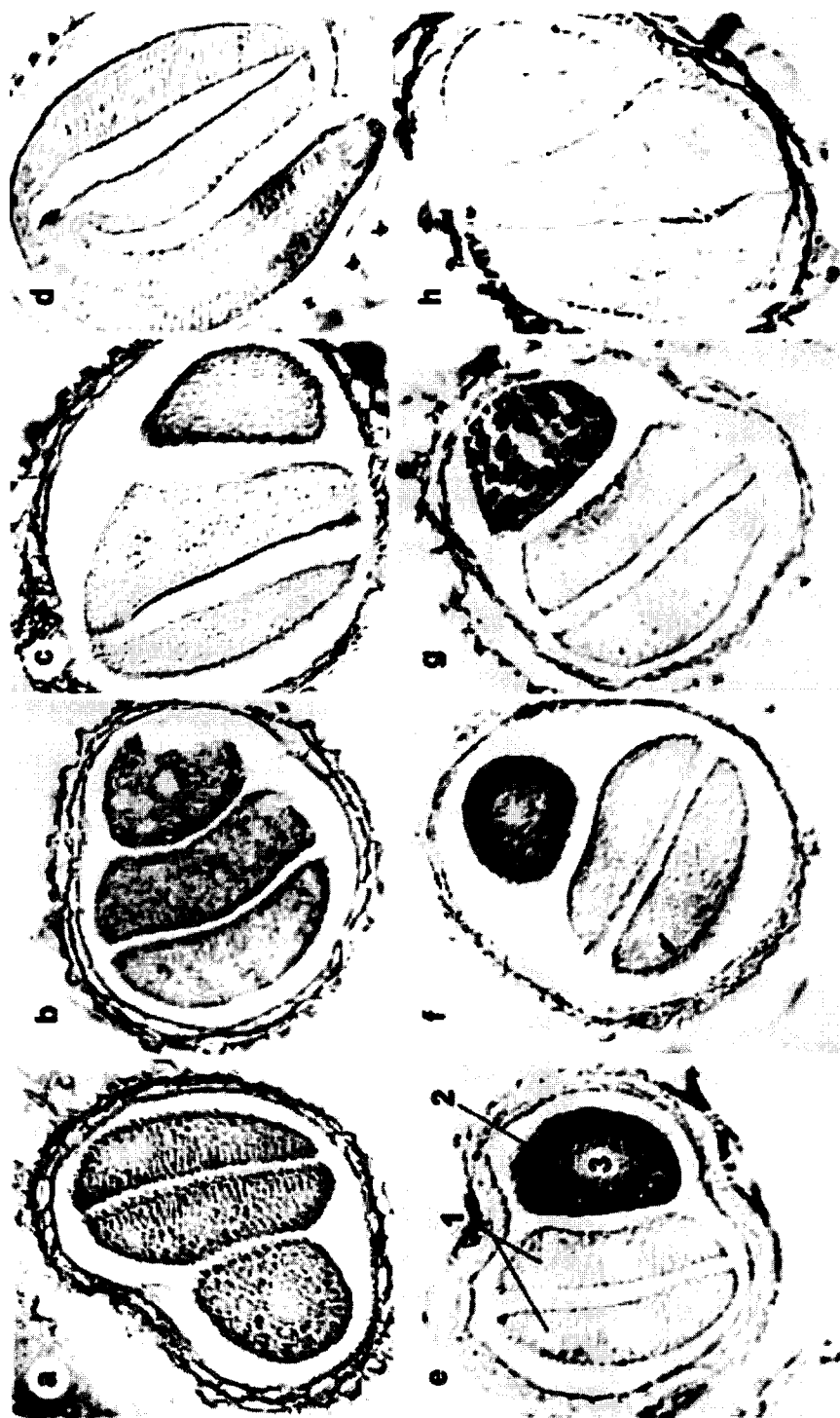


Figure 6. *In situ* localization of *At2S2* and *At2S1* mRNAs. Paraffin-embedded sections of wild-type Landsberg erecta (a and e), *abi3-7* (b and f), *abi3-3* (c and g) and *abi3-3* (h) seeds harvested at 15–16 DAP were hybridized with DIG-labelled antisense probes specific for *At2S2* (top panel) or *At2S1* (bottom panel). 1, cotyledon; 2, embryo axis; 3, provascular tissue.



Figure 7. Immunoblot analysis of AtEm protein content. A 12% SDS-polyacrylamide gel was loaded with 10 μ g (lanes 1 and 2) or 15 μ g (lanes 3, 4 and 5) of protein from wild-type Landsberg erecta (lanes 1 and 5), *abi3-1* (lane 2), *abi3-7* (lane 3) and *abi3-4* (lane 4) mature seeds. Immunological detection was performed with an immune serum that reacts with both the AtEm1 (predicted molecular mass 16.6 kDa) and AtEm6 (predicted molecular mass 9.9 kDa) proteins (Bies *et al.*, 1998).

ble for the lower global content of *At2S2* mRNA in *abi3-7* as compared to *abi3-1*.

As for *At2S2*, the content of *At2S1* mRNA was lower in *abi3-7* seeds compared to *abi3-1* and more *At2S1* mRNA was present in *abi3-7* seeds than in *abi3-4* (Figure 5). In wild-type embryos, the *At2S1* mRNA accumulates almost exclusively in the embryo axis (Figure 6c). In *abi3-1* and *abi3-7* seeds, the *At2S1* expression pattern was essentially identical to that in the wild type (Figure 6f and g). Hence, unlike for *At2S2*, the Ala-458-to-Thr substitution of *abi3-7* appears to have no or very little effect on the spatial regulation of the *At2S1* gene. This additional mutation seems more likely to result in an overall reduction of *At2S1* promoter activity. In *abi3-3*, as in *abi3-4*, no *At2S1* mRNA was detected either by northern blot analysis (Nambara *et al.*, 1992) or by *in situ* hybridization (Figure 6h).

Expression of the AtEm1 and AtEm6 late embryogenesis-abundant (LEA) genes

ABI3 has been shown to tightly regulate the expression of the *Em* genes (*AtEm1* and *AtEm6*) which encode homologues of the 'early methionine-labelled' (Em) LEA protein of wheat (Finkelstein, 1993; Gaubier *et al.*, 1993; Parcy *et al.*, 1994).

Western blot analysis showed that the AtEm6 protein was slightly less abundant in *abi3-1* seeds than in wild type (Figure 7). In contrast, only traces of this protein could be detected in extracts from *abi3-7* seeds and it is not detectable in *abi3-4* protein extract (Figure 7). Northern blot analysis indicated that the lower level of AtEm6 protein in *abi3-1* is correlated with a reduced *AtEm6* mRNA content and that almost no *AtEm6* mRNA is accumulated in *abi3-7* and *abi3-4* seeds (Figure 7). Upon *in situ* hybridization analysis, the *AtEm6* mRNA was found to be uniformly expressed throughout wild-type embryos (Figure 8a)

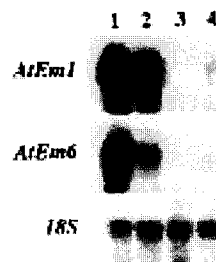


Figure 8. Northern blot analysis of *AtEm1* and *AtEm6* mRNA expression. 10 μ g of total RNA isolated from mature seeds (20 DAP) of wild-type Landsberg erecta (lane 1), *abi3-1* (lane 2), *abi3-5* (lane 3) and *abi3-7* (lane 4) were hybridized with *AtEm1* and *AtEm6* gene-specific probes and with a 18S rRNA probe.

and undetectable in *abi3-7* embryos (Figure 8b). The differences observed between *abi3-1* and *abi3-7* seeds indicate that the Ala-458-to-Thr substitution in *abi3-7* markedly inhibits the developmental regulation of *AtEm6* expression.

The AtEm1 protein was immunologically undetectable in seeds of the severe *abi3-4* mutant (Figure 7). Unlike AtEm6, the abundance of AtEm1 was only slightly reduced in *abi3-7* seeds as compared to wild type and *abi3-1* (Figure 7). Northern blot analysis nevertheless revealed that the level of *AtEm1* mRNA is considerably lower in *abi3-7* than in *abi3-1* seeds (Figure 8). This apparent discrepancy can be resolved since both *AtEm1* mRNA and protein are very stable and a small amount of mRNA might still be sufficient to allow for an almost normal accumulation of protein. By *in situ* hybridization we observed that in wild-type embryos the *AtEm1* mRNA is primarily detected in the bundles of cotyledon vascular tissue and in the provascular tissue of the embryo axis (Figure 8c). In *abi3-7* seeds, in contrast, the *AtEm1* mRNA was only detected at the apical extremities of the provascular tissue of the embryo axis, no signal was detected in the provascular tissue of the central part of the embryo axis (Figure 8d). This observation indicates that, as described above for *At2S2*, the Ala-458-to-Thr substitution in *abi3-7* probably results in a restriction of the spatial distribution of *AtEm1* mRNA expression.

Discussion

In the present report we describe a new *abi3* allele (*abi3-7*) which is intermediate in many aspects of its phenotype between the previously described leaky *abi3-1* and the severe *abi3-4* and *abi3-5* alleles. This

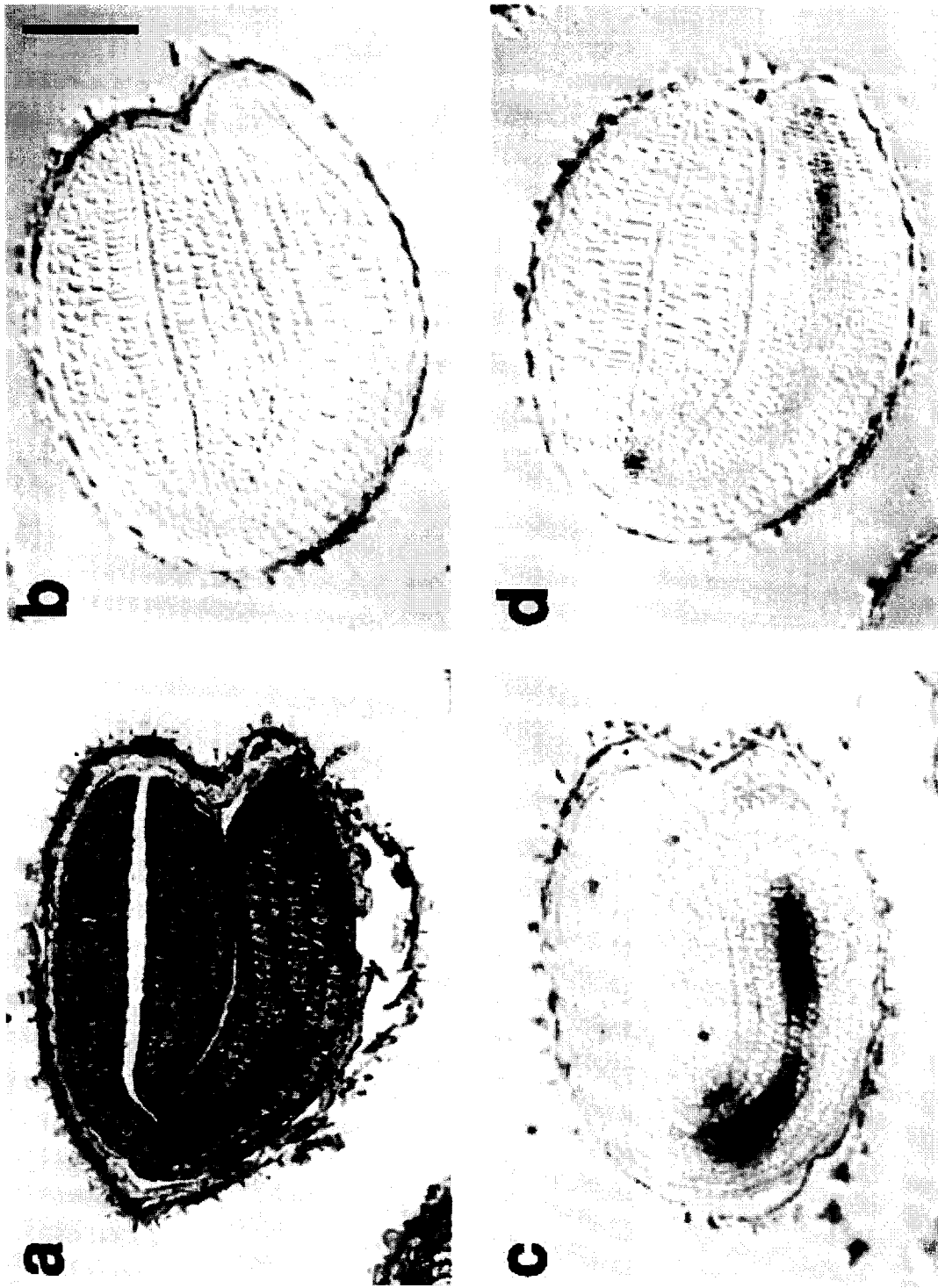


Figure 9. *In situ* localization of *AtEm1* and *AtEm6* mRNAs. Paraffin-embedded sections of mature wild-type *Landsberg erecta* (a and c) and *abi3-7* seeds (b and d) were hybridized with D(G)-labelled anti-sense probes specific for *AtEm6* (top panel) or *AtEm1* (bottom panel).

intermediate position is shown by its insensitivity to ABA, germination speed, seed colour, seed storability and heat stability of the protein and hydrogen bonding interactions as measured by *in situ* Fourier infra-red micro-spectrophotometry (Wolkers *et al.*, 1998). *abi3-7* contains two point mutations in the *ABI3* gene: the *abi3-1* mutation that converts Asp-580 to Asn in the conserved B3 domain, and a novel mutation that converts Ala-458 to Thr in the conserved B2 domain. *abi3-7* phenotypes most likely result from the combined effects of these two mutations. Nevertheless, comparative analysis of the *abi3-1* and *abi3-7* mutants provides first indications on the phenotypic consequences of a point mutation in the conserved B2 domain of the ABI3 protein. Analysis of protein extracts with an anti-ABI3 immune serum showed that the abundance of the ABI3 protein is similar in *abi3-7* and in *abi3-1* seeds. This indicates that the phenotypic differences between *abi3-1* and *abi3-7* result primarily from the direct impact of the Ala-458-to-Thr substitution on the functional properties of the ABI3 protein, rather than from an indirect effect of this mutation on the steady-state level of ABI3 protein.

Whereas the level of the AtEm6 protein is slightly reduced in *abi3-1* seeds compared to wild type, expression of AtEm6 was undetectable in *abi3-7* seeds both at the protein and mRNA levels indicating that the B2 domain of the ABI3 protein plays a critical role in the regulation of *AtEm6* during seed development. This is consistent with the observation that in transient expression studies, the B2 domain of the maize VP1 protein is required for transactivation of the wheat *Em* gene (Hill *et al.*, 1996). Because this VP1 activation requires the G-box-type element Em1a (GACACGTGGC) of the wheat *Em* promoter (Guiltinan *et al.*, 1990; Vasil *et al.*, 1995) and because the *Arabidopsis AtEm6* promoter contains a sequence that perfectly matches this motif (Gaubier *et al.*, 1993) it would be interesting to investigate whether the Em1a motif of *AtEm6* is involved in the regulation by ABI3.

The *abi3-7* mutation in the B2 domain had essentially no effect on the expression of *At2S1* but markedly restricted the spatial distribution of *At2S2* mRNA. Remarkably, the expression pattern of *At2S2* in *abi3-7* embryos was comparable to that of *At2S1* in the wild type. While *At2S2* mRNA was still present in the axis of *abi3-7* embryos, no signal was detected in the cotyledons indicating that the B2 domain of ABI3 is essential for expression of *At2S2* in the cotyledons. The analysis of hybrid promoter constructs between *At2S1* and *At2S2* pointed to the sequence CATGCA

as being likely involved in the expression of *At2S2* in cotyledons (Da Silva Conceicao and Krebbers, 1994). Intriguingly, this sequence resembles the core TC-CATGCAT motif of the Sph element that mediates the activation of the maize *C1* anthocyanin regulatory gene by VP1 (Hattori *et al.*, 1992; Kao *et al.*, 1996). Recent studies support the hypothesis that the conserved B3 domain of VP1 is essential for this transactivation (Carson *et al.*, 1997; Suzuki *et al.*, 1997).

The Ala-458-to-Thr substitution in the B2 domain of ABI3 markedly inhibited *AtEm1* expression in the longitudinal central part of the provascular tissues of the embryo axis, but not at the apical extremity of the embryo axis. As for *At2S2* gene regulation, different *cis*-acting sequences interacting directly or indirectly with ABI3 might be responsible for *AtEm1* gene expression in different tissues. Another possibility could be that in each tissue ABI3 interacts with tissue-specific co-factors, and that these interactions are differentially sensitive to a given mutation in the ABI3 protein and/or involve different domains of this protein.

In conclusion, the present study provides genetic evidence that the conserved B2 domain of the ABI3 protein is critical for the developmental regulation of *AtEm6* and (at least in certain tissues of the developing embryo) of *At2S2* and *AtEm1*. It has been shown that the B2 domain of the maize VP1 protein enhances the *in vitro* DNA binding activity of diverse transcription factors (Hill *et al.*, 1996), but whether this property contributes to VP1 action *in vivo* remains unclear. Further work is clearly needed to establish the biochemical activity of the B2 domain in its native protein context. The identification of all the factors interacting with each domain of the ABI3 protein and all the *cis*-acting motif elements involved in the regulation by ABI3 are also necessary to completely understand how ABI3 and its homologues are able to regulate the expression of so many genes during seed development and maturation. According to our data specific domains of ABI3 or some tissue-specific co-factors interacting with ABI3 might be involved in the regulation of a same gene in the different tissues of the embryo.

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